IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Fabrice Le Gall et al.	Art Unit: 1644
Application No. 10/527,346	Confirmation No.: 7228
Filed: September 23, 2005	Examiner: SKELDING, Zachary S.
For: HUMAN CD3-ANTIBODY WITH IMMUNOSUPPRESSIVE PROPERTIES	Attorney Docket: 03528.0146.PCUS00

RULE 132 DECLARATION OF DR. MELVYN LITTLE

I, Melvyn Little, Ph.D., Chief Scientific Officer at Affimed Therapeutics AG, hereby declare as follows:

1. I received a Ph.D. degree in biochemistry from University College of North Wales – Bangor, United Kingdom. After postdoctoral work on the mechanism of action of estradiol at the Max-Planck-Institute for Cell Biology in Wilhelmshaven, Germany, I joined the scientific staff of the German Cancer Research Center (DKFZ) in Heidelberg and habilitated at the Faculty of Biology, University Heidelberg. In 1990, I became head of the "Recombinant Antibodies" research group at the DKFZ.

2. I founded Affimed Therapeutics AG in 2000, which is the assignee of the above-referenced patent application. I currently serve as Chief Scientific Officer (CSO) at Affimed Therapeutics AG.

3. I am a co-inventor of the invention claimed in the above-referenced patent application.

4. I have reviewed the Office Action dated October 3, 2008 and the Final Office Action dated July 9, 2009 for the above-referenced patent application, in which the Examiner rejected the claims as being unpatentable over Smith et al. (WO 9847531) in view of Hsu et al. (Transplantation. 1999 Aug 27; 68(4): 545-54), Holliger et al. (US Pat. No. 5,837,242) and Chapman et al. (Nat Biotechnol., 1999; 17(8): 780-3), as well as being unpatentable over Smith et al., in view of Hsu et al., Holliger et al., and Chapman et al, and further in view of Kipriyanov et al. (Protein Eng., 1997; 10(4): 445-53).

5. I am a co-author of Kipriyanov et al. (Protein Eng., 1997; 10(4): 445-53).

6. I supervised the following scientific experiments, which demonstrate the superior property of the antibody claimed in the above-referenced patent application. In particular, the following experiments compared T cell proliferation between a human anti-CD3 diabody (i.e., $scFv_6$) according to the above-referenced patent application and a human tandem diabody (i.e., TandAb) containing the same variable domains as the diabody ($scFv_6$). Both antibodies are devoid of any constant domains. The structure of TandAb is described in detail in Kipriyanov, et al., J. Mol. Biol. (1999) (copy enclosed herewith).

Experiment A. Proliferation of human PBMC in the presence of anti-human CD3 antibodies

Materials and Methods

To determine whether the bivalent human diabody ($scFv_6$) or the tetravalent human TandAb antibody stimulate freshly isolated peripheral blood mononuclear cells (PBMC) to proliferate, a MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was performed on cultures of human PBMC in the presence of increasing concentrations of the diabody, TandAb, buffer (20 mM Tris, pH 7.5) and, as a positive control, murine IgG anti-CD3 OKT3.

PBMCs were isolated from heparinized peripheral blood of a healthy volunteer by density gradient centrifugation. The blood sample was diluted with a two-fold volume of PBS (Gibco), layered on a cushion of Histopaque-1077 (Sigma) and centrifuged at 800 g for 25 min. PBMC located in the interface were collected and washed 3 times with PBS before they were resuspended in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine and 100 IU/ml penicillin G sodium and 100 µg/mL streptomycin sulfate (herein referred to as RPMI medium; all components from Invitrogen) and used in the proliferation assay.

2×10^5 PBMC were seeded in individual wells of a flat-bottom microplate together with increasing concentrations of the indicated antibodies or buffers in a total volume of 160 µl/well. After 5 days of incubation in a humidified incubator with 5% CO_2 at 37°C 24 µl/well dye solution (Promega) was added and the plate was incubated for further 3 hours until a strong color development was observed. In order to solubilize the formazan crystals, 160 µl/well of the stop/solubilization solution (Promega) was added to the wells, mixed and incubated for additional 2 hours. The absorbance of the samples was measured at 570 nm with microplate reader (Victor 3, Perkin Elmer) and the absorbance at the reference

wavelength at 650 nm was subtracted. Mean and standard deviation of triplicates were plotted in a diagram using the GraphPad Prism software.

Results

Fig. 1 shows a comparison of immunosuppression effects of the monoclonal antibody OKT3, a tetravalent humanized anti-CD3 in the tandem diabody (TandAb) format (Kipriyanov et al. J. Mol. Biol. (1999) 293, 41-56), which is devoid of constant domains, and the diabody according to the above-referenced patent application comprising the same humanized anti-CD3 domains as TandAb. The results demonstrated that both OKT3 and TandAb induced a significant proliferation of PBMC at antibody concentrations between 1 pM and 1 nM. In contrast, the anti-CD3 diabody according to the above-referenced patent application induced no significant proliferation at the same concentration range or higher concentrations up to 0.1 μM. The buffer control neither induced nor inhibited PBMC proliferation.

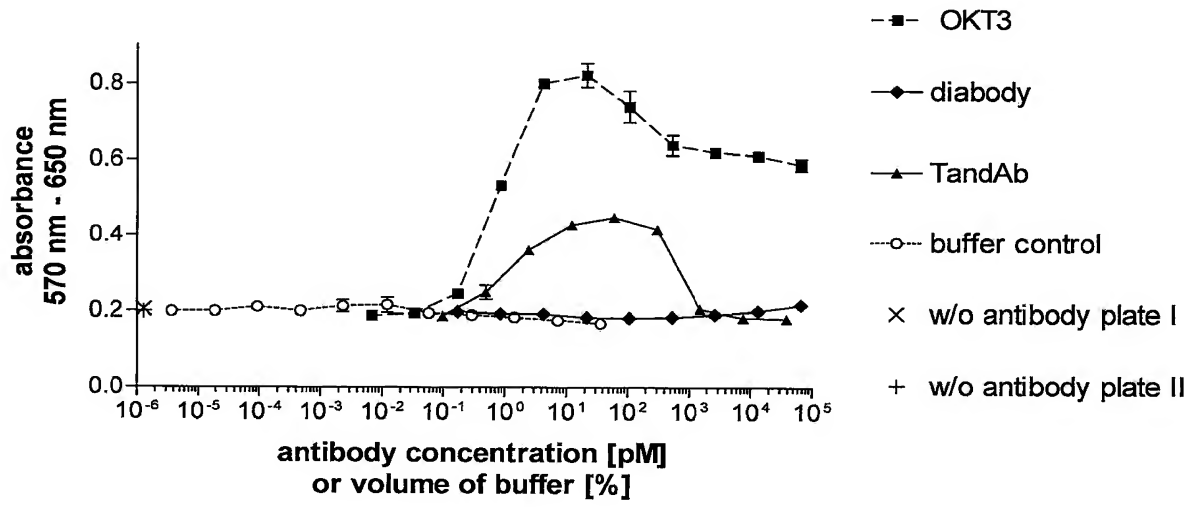

Fig. 1: Proliferation of human PBMC in the presence of anti-human CD3 antibodies. 2 x 10^5 PBMC were seeded in individual wells of a 96-well micro plate in the presence of the indicated concentrations of murine anti-CD3 antibody OKT3, human diabody $scFv_6$, human anti-CD3 TandAb or 20 mM Tris, pH 7.5 as a buffer control. After 5 days incubation at 37°C in a humidified incubator with 5% CO_2 the relative amount of living cells was determined with an MTT assay. Mean and SD absorbance values of triplicates are plotted in the diagram.

Experiment B. Modulation of T cell receptor complex on Jurkat cells by human anti-CD3 antibodies devoid of constant domains

Materials and Methods

To determine whether the bivalent human diabody ($scFv_6$) or the tetravalent human tandem diabody (TandAb) induce internalization of the T cell receptor complex (TCR) on Jurkat cells of a human acute T cell leukemia cell line, a TCR Modulation test was performed on cultures of Jurkat cells in the presence of increasing concentrations of the diabody, TandAb and, as a positive control, murine IgG anti-CD3 OKT3.

1 x 10^6 Jurkat cells were seeded in individual wells of a round-bottom microplate together with increasing concentrations of the indicated antibodies in a total volume of 200 µl/well. After 18 h of incubation in a humidified incubator with 5% CO_2 at 37°C cells were washed, stained with PC5-conjugated anti-TCRα/β antibody and analyzed by flow cytometry (Beckman Coulter FC500 MPL). Mean fluorescence intensities were plotted in a diagram using the GraphPad Prism software.

Results

Fig. 2 shows complete internalization of the TCR complex by all tested antibodies at concentrations higher than 2 nM. The EC_{50} values for all three antibodies were very similar between 350 pM and 590 pM.

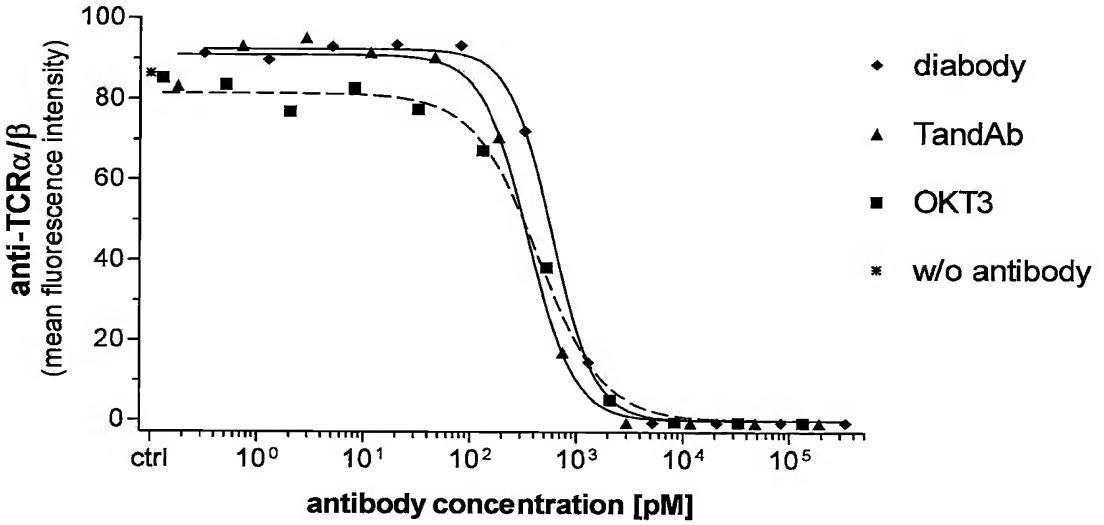

Fig. 2: Modulation of T-Cell receptor complex on Jurkat cells in the presence of anti-human CD3 antibodies. 1 x 10^6 Jurkat cells were seeded in individual wells of a 96-well micro plate in the presence of the indicated concentrations of murine anti-CD3 antibody OKT3, human diabody $scFv_6$ or human anti-CD3 TandAb. After 18 h incubation at 37°C in a humidified incubator with 5% CO_2 cells were stained with PC5-conjugated anti-TCRα/β antibody and analyzed by flow cytometry.

Conclusion

The above experiments demonstrate that an anti-CD3 diabody according to the above-referenced patent application did not induce any T cell proliferation, whereas a tandem diabody TandAb induced a significant T cell response. It is noted that both antibodies are devoid of constant domains and are composed of the identical CD3 specific domains. That is, it is unexpected that an anti-CD3 diabody according to the above-referenced patent application exhibited a significant immunosuppression effect, and that such effect was superior over prior art-known diabody TandAb.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 10th Dec. 2009

Dr. Melvyn Little
Chief Scientific Officer
Affimed Therapeutics AG